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(21) International Application Number: PCT/EP94/01362 (22) International Filing Date: 29 April 1994 (29.04.94) (30) Priority Data: 93 107 089.0 30 April 1993 (30.04.93) EP (34) Countries for which the regional or international application was filed: DE et al. 93 107 849.7 13 May 1993 (13.05.93) EP (34) Countries for which the regional or international application was filed: DE et al. (71) Applicant (for all designated States except US): BIOGNOSTIK GESELLSCHAFT FÜR BIOMOLEKULARE DIAGNOSTIK MBH [DE/DE]; Carl-Giesecke-Strasse 3, D-37079 Göttingen (DE). (72) Inventors; and (75) Inventors/Applicants (for US only): SCHLINGENSIEPEN, Georg-Ferdinand [DE/DE]; Am Goldgraben 20, D-37073 Göttingen (DE). BRYSCH, Wolfgang [DE/DE]; Am Goldgraben 2, D-37073 Göttingen (DE). SCHLINGENSIEPEN, Karl-Hermann [DE/DE]; Bovender Strasse 5, D-37120 Bovenden (DE). SCHLINGENSIEPEN, Reimar [DE/DE]; Am Goldgraben 13, D-37073 Göttingen (DE). BOGDAHN,		Ulrich [DE/DE]; Mittlere Heerbergstrasse 25a, D-97078 Würzburg (DE). (74) Agents: MEYERS, Hans-Wilhelm et al.; Deichmannhaus am Hauptbahnhof, D-50667 Köln (DE). (81) Designated States: AU, CA, CN, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published Without international search report and to be republished upon receipt of that report.	
(54) Title: ANTISENSE-OLIGONUCLEOTIDES FOR THE TREATMENT OF IMMUNOSUPPRESSIVE EFFECTS OF TRANSFORMING GROWTH FACTOR- β (TGF- β)			
(57) Abstract <p>Antisense-oligonucleotides or effective derivatives thereof hybridizing with an area of a gene coding for transforming growth factor-β (TGF-β) comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID No. 1 - 56 and 137 or comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID No. 57 to 136 each of the nucleic acids having a DNA- or RNA-type structure.</p>			

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Antisense-oligonucleotides for the treatment of immuno-suppressive effects of transforming growth factor- β (TGF- β)

The present invention is related to antisense-oligonucleotides or effective derivatives thereof hybridizing with an area of a gene coding for transforming growth factor- β (TGF- β), oligonucleotides as nonsense control nucleotides, a pharmaceutical composition comprising at least one antisense-oligonucleotide or effective derivatives thereof hybridizing with an area of a gene coding for TGF- β as well as a use of antisense-oligonucleotides for the manufacturing of a pharmaceutical composition for the treatment of tumors and/or the treatment of the immunosuppressive effect of TGF- β .

The transforming growth factor- β (TGF- β) is a factor which is, for example, secreted by human glioma cells. Human gliomas such as glioblastoma are human tumors for which at present no satisfactory therapy exists. The TGF- β supports in an autocrine manner the growing of the respective tumor cells. The factor shows immunosuppressive effects and reduces (the proliferation of such cytotoxic T-lymphocytes which otherwise would be able to destroy the glioma cells.

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The suppression of immune responsiveness has been well documented in patients with malignant gliomas. These patients express a variety of immunological deficiencies including cutaneous anergy, depressed antibody production, diminished numbers of circulating T-cells (Brooks, W. H., Netsky, M. G., Horwitz, D. A., Normansell, D. E. Cell mediated immunity in patients with primary brain tumors, J. Exp. Med., 136: 1931 - 1947, 1972 and Roszman, T., Elliott, L., Brooks, W. Modulation of T-cell function by gliomas, Immunol. Today 12: 370 - 374, 1991). More recent studies indicate that these impairments may result from malfunctions in physiological pathways required for normal T-cell activation and from quantitative and qualitative defects in T-cell subsets.

In Proceedings of the 82nd Annual meeting of the American Association for Cancer Research, Houston Texas, USA, May 15 - 18, 1991, Proc AM ASSOC CANCER RES ANNU MEET 32 (O), 1991,, 427 is disclosed that factor- β -antisense-oligonucleotides inhibit a human melanoma cell line under serum-enriched and stimulate under serum-free culture conditions. The results established indicate different roles of cellular TGF- β_1 in the growth regulation of HTZ-19-cells depending on the amount of serum present in the culture medium. In addition this may indicate the biological potential and possible draw-backs of exogenously administered TGF- β -antisense.

J. EXP. MED. 174 (4), 1991, 925 - 930, Hatzfield J. et al, "Release of early human hematopoietic progenitors from quiescence by antisense transforming growth factor β -1 or Rb oligonucleotides" discloses release of early human hematopoietic progenitors from quiescence by antisense transforming growth factor β_1 or Rb oligonucleotides. Rb antisense TGF- β negatively regulates the cycling status of early hematopoietic progenitors through interaction with the Rb gene product.

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Proceedings of the National Academy of Sciences of USA, Vo. 88, February 1991, Washington US, pages 1516 - 1520, Potts, J. et al., "Epithelial-mesenchymal transformation of embryonic cardiac antisense oligodeoxynucleotide to transforming growth factor beta 3'" discloses that epithelial-mesenchymal transformation of embryonic cardiac endothelial cells is inhibited by a modified antisense oligodeoxynucleotide to transforming growth factor $\beta 3$. The transformation depends on the activity of a transforming growth factor β (TGF- β) molecule produced by the heart. Modified antisense oligodeoxynucleotides generated to non-conserved regions of TGF- $\beta 1$, -2, -3 and -4 were prepared in order to examine the possible roles of these members in this transformation. As a result it has been shown that a specific member of the TGF- β family (TGF- $\beta 3$) is essential for the epithelial-mesenchymal transformation.

WO-A 92/17206 discloses a composition for use in the treatment of wounds to inhibit scar tissue formation during healing comprising an effective activity-inhibitor amount of a growth factor neutralising agent or agents specific against only fibrotic growth factors together with a pharmaceutically acceptable carrier. The method of preparation of said composition and method of administering the composition to a host suffering from tissue wounding is also disclosed.

WO-A 90/09180 discloses methods useful in autologous bone marrow transplantation and cancer therapy. Bone marrow cells from a patient having cancer are treated with selected antisense oligonucleotides in order to deplete the bone marrow of malignant cells prior to infusion back into the bone marrow donor.

It is an object of the present invention to provide a method for the treatment of cancer cells which are correlated with an immunosuppression. Another object of the present invention

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is to provide an effective agent which inhibits the growth of tumor cells which are related to an immunosuppression.

According to the invention antisense-oligonucleotides or effective derivatives thereof which hybridizes with an area of gene region coding for transforming growth factor- β (TGF- β) comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID NO. 1 - 56 and 137 or comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID NO. 57 to 136 each of the nucleic acids having a DNA- or RNA-type structure are able to solve the problems addressed above. Preferably, the antisense-oligonucleotides hybridize with an area of a gene region coding for growth factor- β_1 , - β_2 and/or β_3 . The antisense-oligonucleotide is either able to hybridize with areas of a gene region coding for TGF- β and/or areas of a gene region coding and non coding for TGF- β . For example, some nucleotides of the antisense-oligonucleotide sequence hybridizing with an area of a gene region coding for transforming growth factor- β is hybridizing with an area which does not code for the transforming growth factor whereas, the other part of the respective sequence does hybridize with a gene region coding for TGF- β . Of course, it is also in the scope of the present invention that the antisense-oligonucleotide hybridizes with an area of a gene region just coding for growth factor- β . It is also understood by the skilled person that fragments having subsequences of the antisense-oligonucleotide works according to the invention so long as production of TGF- β is reduced or inhibited.

In a preferred embodiment of the present invention the antisense-oligonucleotide or effective derivative thereof is a phosphorothioate-oligodeoxynucleotide.

According to the invention the antisense-oligonucleotides are obtainable by solid phase synthesis using phosphite triester chemistry by growing the nucleotide chain in 3'-5'

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direction in that the respective nucleotide is coupled to the first nucleotide which is covalently attached to the solid phase comprising the steps of

- cleaving 5'DMT protecting group of the previous nucleotide,
- adding the respective nucleotide for chain propagation,
- modifying the phosphite group subsequently cap unreacted 5'-hydroxyl groups and
- cleaving the oligonucleotide from the solid support,
- followed by working up the synthesis product.

The chemical structures of oligodeoxy-ribonucleotides are given in figure 1 as well as the respective structures of antisense oligo-ribonucleotides are given in figure 2. The oligonucleotide chain is to be understood as a detail out of a longer nucleotide chain.

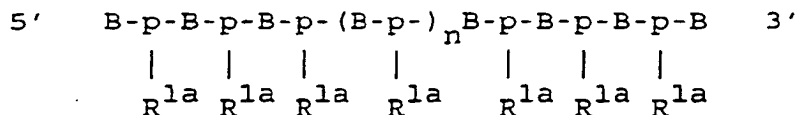
In figure 1 lit. B means an organic base such as adenine (A), guanine (G), cytosine (C) and thymine (T) which are coupled via N9(A,G) or N1(D,T) to the desoxyribose. The sequence of the bases is the reverse complement of the genetic target sequence (mRNA-sequence). The modifications used are

1. Oligodeoxy-ribonucleotides where all R¹ are substituted by

- 1.1 R¹ = O
- 1.2 R¹ = S
- 1.3 R¹ = F
- 1.4 R¹ = CH₃
- 1.5 R¹ = OEt

2. Oligodeoxy-ribonucleotides where R¹ is varied at the internucleotide phosphates within one oligonucleotide

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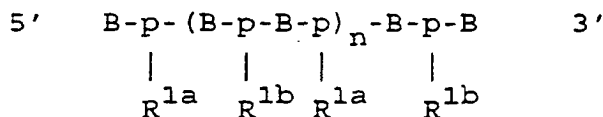
where B = deoxy-ribonucleotide dA, dC, dG or dT depending
on gene sequence

p = internucleotide phosphate

n = an oligodeoxy-ribonucleotide stretch of length
6 - 20 bases

- | | | |
|-----|------------------|-----------------|
| 2.1 | $R^{1a} = S;$ | $R^{1b} = O$ |
| 2.2 | $R^{1a} = CH_3;$ | $R^{1b} = O$ |
| 2.3 | $R^{1a} = S;$ | $R^{1b} = CH_3$ |
| 2.4 | $R^{1a} = CH_3;$ | $R^{1b} = S$ |

3. Oligodeoxy-ribonucleotides where R^1 is alternated at
the internucleotide phosphates within one oligonucleo-
tide



where B = deoxy-ribonucleotide dA, dC, dG or dT
depending on gene sequence

p = internucleotide phosphate

n = an oligodeoxy-ribonucleotide stretch of
length 4 - 12 dinucleotides

- | | | |
|-----|------------------|-----------------|
| 3.2 | $R^{1a} = S;$ | $R^{1b} = O$ |
| 3.2 | $R^{1a} = CH_3;$ | $R^{1b} = O$ |
| 3.3 | $R^{1a} = S;$ | $R^{1b} = CH_3$ |

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4. Any of the compounds 1.1 - 1.5; 2.1 - 2.4; 3.1 - 3.3 coupled at R^2 with the following compounds which are covalently coupled to increase cellular uptake

- 4.1 cholesterol
- 4.2 poly(L)lysine
- 4.3 transferrin

5. Any of the compounds 1.1 - 1.5; 2.1 - 2.4; 3.1 - 3.3 coupled at R^3 with the following compounds which are covalently coupled to increase cellular uptake

- 5.1 cholesterol
- 5.2 poly(L)lysine
- 5.3 transferrin

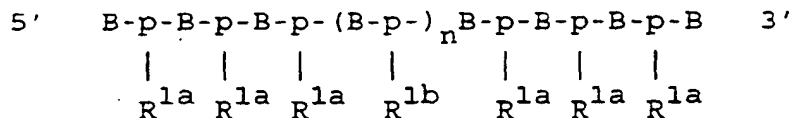
In the case of the RNA-oligonucleotides (figure 2) are the basis (adenin (A), guanin (G), cytosin (C), uracil (U)) coupled via N9 (A,G) or N1 (C,U) to the ribose. The sequence of the basis is the reverse complement of the genetic target sequence (mRNA-sequence). The modifications in the oligonucleotide sequence used are as follows

6. Oligo-ribonucleotides where all R^1 are substituted by

- 6.1 $R^1 = O$
- 6.2 $R^1 = S$
- 6.3 $R^1 = F$
- 6.4 $R^1 = CH_3$
- 6.5 $R^1 = OEt$

7. Oligo-ribonucleotides where R^1 is varied at the internucleotide phosphates within one oligonucleotide

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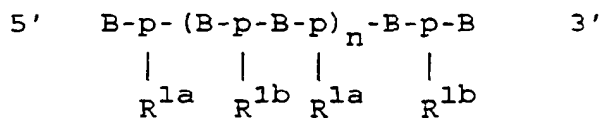
where B = ribonucleotide dA, dC, dG or dT depending
on gene sequence

p = internucleotide phosphate

n = an oligo-ribonucleotide stretch of length 4 -
20 bases

- | | | |
|-----|------------------|-----------------|
| 7.1 | $R^{1a} = S;$ | $R^{1b} = O$ |
| 7.2 | $R^{1a} = CH_3;$ | $R^{1b} = O$ |
| 7.3 | $R^{1a} = S;$ | $R^{1b} = CH_3$ |
| 7.4 | $R^{1a} = CH_3;$ | $R^{1b} = S$ |

8. Oligo-ribonucleotides where R^1 is alternated at the
internucleotide phosphates within one oligonucleotide



where B = ribonucleotide dA, dC, dG or dT depending
on gene sequence

p = internucleotide phosphate

n = an oligo-ribodinucleotide stretch of length
4 - 12 dinucleotides

- | | | |
|-----|------------------|-----------------|
| 8.2 | $R^{1a} = S;$ | $R^{1b} = O$ |
| 8.2 | $R^{1a} = CH_3;$ | $R^{1b} = O$ |
| 8.3 | $R^{1a} = S;$ | $R^{1b} = CH_3$ |

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9. Any of the compounds 6.1 - 6.5; 7.1 - 7.4; 8.1 - 8.3 coupled at R^2 with the following compounds which are covalently coupled to increase cellular uptake

- 9.1 cholesterol
- 9.2 poly(L)lysine
- 9.3 transferrin

10. Any of the compounds 6.1 - 6.5; 7.1 - 7.4; 8.1 - 8.3 coupled at R^3 the following compounds are covalently coupled to increase cellular uptake

- 10.1 cholesterol
- 10.2 poly(L)lysine
- 10.3 transferrin

11. Any of the compounds 6.1 - 6.5; 7.1 - 7.4; 8.1 - 8.3; 9.1 - 9.3; 10.1 - 10.3 where all R^4 are substituted by

- 11.1 $R^4 = O$
- 11.2 $R^4 = F$
- 11.3 $R^4 = CH_3$

Modifications of the antisense-oligonucleotides are advantageous since they are not as fast destroyed by endogeneous factors when applied as this is valid for naturally occurring nucleotide sequences. However, it is understood by the skilled person that also naturally occurring nucleotides having the disclosed sequence can be used according to the invention. In a very preferred embodiment the modification is a phosphorothioat modification.

The synthesis of the oligodeoxy-nucleotide of the invention is described as an example in a greater detail as follows.

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Oligodeoxy-nucleotides were synthesized by stepwise 5' addition of protected nucleosides using phosphite triester chemistry. The nucleotide A was introduced as 5'-dimethoxytrityl-deoxyadenosine (N^4 -benzoyl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite (0.1 M); C was introduced by a 5'-dimethoxytrityl-deoxycytidine (N^4 -benzoyl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite; G was introduced as 5'-dimethoxytrityl-deoxyguanosine (N^8 -isobutyryl)-N,N'-diisopropyl-2-cyanoethyl phosphoramidite and the T was introduced as 5'-dimethoxytrityl-deoxythymidine-N,N'-diisopropyl-2-cyanoethyl phosphoramidite. The nucleosides were preferably applied in 0.1 M concentration dissolved in acetonitrile.

Synthesis was performed on controlled pore glass particles of approximately 150 μ m diameter (pore diameter 500 Å) to which the most 3' nucleoside is covalently attached via a long-chain alkylamin linker (average loading 30 μ mol/g solid support).

The solid support was loaded into a cylindrical synthesis column, capped on both ends with filters which permit adequate flow of reagents but hold back the solid synthesis support. Reagents were delivered and withdrawn from the synthesis column using positive pressure of inert gas. The nucleotides were added to the growing oligonucleotide chain in 3' \rightarrow 5' direction. Each nucleotide was coupled using one round of the following synthesis cycle:

cleave 5' DMT (dimethoxytrityl) protecting group of the previous nucleotide with 3-chloroacetic acid in dichloromethane followed by washing the column with anhydrous acetonitrile. Then simultaneously one of the bases in form of their protected derivative depending on the sequence was added plus tetrazole in acetonitrile. After reaction the reaction mixture has been withdrawn and the phosphite was oxidized with a mixture of sulfur (S_8) in carbon disulfide/pyridine/triethylamine. After the oxidation reaction the

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mixture was withdrawn and the column was washed with acetonitrile. The unreacted 5'-hydroxyl groups were capped with simultaneous addition of 1-methylimidazole and acetic anhydride/lutidine/tetrahydrofuran. Thereafter, the synthesis column was washed with acetonitrile and the next cycle was started.

The work up procedure and purification of the synthesis products occurred as follows.

After the addition of the last nucleotide the deoxynucleotides were cleaved from the solid support by incubation in ammonia solution. Exocyclic base protecting groups were removed by further incubation in ammonia. Then the ammonia was evaporated under vacuum. Full-length synthesis products still bearing the 5'-DMT protecting group were separated from shorter failure contaminants using reverse phase high performance liquid chromatography on silica C₁₈ stationary phase. Eluents from the product peak were collected, dried under vacuum and the 5'-DMT protecting group cleaved by incubation in acetic acid which was evaporated thereafter under vacuum. The synthesis products were solubilized in the deionized water and extracted three times with diethylether. Then the products were dried in vacuo. Another HPLC-AX chromatography was performed and the eluents from the product peak were dialysed against excess of Trisbuffer as well as a second dialysis against deionized water. The final products were lyophilized and stored dry.

The antisense-oligonucleotides of the invention can be used as pharmaceutical composition or medicament. This medicament can be used for treating tumors in which the expression of TGF- β is of relevance for pathogenicity by inhibiting the transforming growth factor- β and thereby reducing an immunosuppression and/or inhibiting pathological angiogenesis. The reduction of immunosuppression caused by the administration of an effective dose of an antisense TGF- β -oligonucleotides

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may be accompanied by an augmented proliferation of cytotoxic lymphocytes in comparison with the status before administration of the medicament. Thereupon, the lymphocytes are starting their cytotoxic activity decreasing the numbers of tumor cells.

The medicament of the present invention is further useful for the treatment of endogeneous hyperexpression of TGF- β , for treatment of rest tumors, for treatment of neurofibroma, malignant glioma including glioblastoma and for the treatment and prophylaxis of skin carcinogenesis as well as treatment of esophageal and gastric carcinomas.

The effect of TGF- β_2 -specific antisense-oligonucleotides on human T cell proliferation and cytotoxicity upon stimulation with autologous cultured glioma cells was investigated. It was demonstrated that TGF- β_2 -derived phosphorothioate-derivatives S-ODN's may specifically inhibit protein expression of TGF- β in glioma cells. In addition, TGF- β_2 -specific S-ODN's revers - to a significant amount - immunosuppressive effects of TGF- β upon T-cell proliferation and cytotoxicity.

It has been shown that T-cell response in human brain tumor patients is clearly reduced and that tumor infiltrating lymphocytes have only marginal impact upon tumor progression of individual patients (Palma, L., Di Lorenzo, N., Guidetti, B. Lymphocytes infiltrates in primary glioblastomas and recidivous gliomas, J. Neurosurg., 49: 854 - 861, 1978 and Ridley, A., Cavanagh, J. B. Lymphocytes infiltration in gliomas, Evidence of possible host resistance. Brain, 4: 117 - 124, 1971). Isolated tumor infiltrating lymphocytes from brain tumors are functionally incompetent, these immunosuppressive effects have been attributed to TGF- β_2 in vitro and in vivo (Bodmer, S., Stromer, K., Frei, K., Siepl, Ch., de Tribolet, N., Heid, I., Fontana, A., Immunosuppression and transforming growth factor- β_2 in glioblastoma, J.

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Immunol., 143: 3222 - 3229, 1989; Couldwell, W. T., Dore-Duffy, P., Apuzzo, M. L. J., Antel, J. P. Malignant glioma modulation of immune function: relative contribution of different soluble factors, J. Neuroimmunol., 33: 89 - 96, 1991; Kuppner, M. C., Hamou, M. F., Sawamura, Y., Bodner, S., de Tribolet, N., Inhibition of lymphocyte function by glioblastoma derived transforming growth factor β_2 , J. Neurosurg., 71: 211 - 217, 1989; Maxwell, M., Galanopoulos, T., Neville-Golden, J., Antoniades, H. N., Effect of the expression of transforming growth factor- β_2 in primary human glioblastomas on immunosuppression and loss of immune surveillance, J. Neurosurg., 76: 799 - 804, 1992; Palladino, M. A., Morris, R. E., Fletscher Starnes, H., Levinson, A. D., The transforming growth factor betas, A new family of immunoregulatory molecules, Ann. N.Y. Acad. Sci., 59: 181 to 187, 1990; Roszman, T., Elliott, L., Brooks, W., Modulation of T-cell function by gliomas, Immunol Today 12: 370 - 374, 1991).

Figure 3: IGF- β western blot analysis of serum free glioma culture cell lysates. Lanes 2 (HTZ-153), 3 (HTZ-209), and 4 (HTZ-243) indicate blots of respective cell lysates with TGF- β_2 specific antibody. Lane 1 represents a TGF- β positive control employing 50 ng pure TGF- β_2 . TGF- β_2 -antisense treated cells are displayed in lanes A. Untreated control cells are depicted in lanes B. Cells were treated with antisense oligonucleotides for 48 hrs (1 μ M final concentration).

Figure 4: IGF- β_1 -mRNA expression in glioma cells. Each lane contained 20 μ g of cytoplasmatic RNA from tumors A (HTZ-153), B (HTZ-209), C (HTZ-243) that hybridized to a 32 P-labeled TGF- β_1 oligonucleotide probe. To verify equal amounts of RNA, the blot was stained with methylene blue prior to hybridization (lanes A', B', C').

Figure 5: TGF- β_2 -mRNA expression in glioma cells. Each lane contained 20 μ g of cytoplasmatic RNA from tumors A (HTZ-153),

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B (HTZ-209), C (HTZ-243) that hybridized to a ^{32}P -labeled TGF- β_2 oligonucleotide probe. To verify equal amounts of RNA, the blot was stained with methylene blue prior to hybridization (A', B', C').

Figure 6: TGF- β_2 -mRNA expression in glioma cells after TGF- β_2 -S-ODN treatment. Cytoplasmatic RNA of untreated glioma cells A (HTZ-153), B (HTZ-209) and C (HTZ-243) or glioma cells A', B' and C' treated for 48 hours with 1 μM (f.c.) TGF- β_2 -specific S-ODN's under serum-enriched culture conditions, was isolated and processed for Northern blot analysis. Each lane contained 20 μg of cytoplasmatic RNA hybridized to a ^{32}P -labeled TGF- β_2 oligonucleotide probe.

Figure 7: Effect of TGF- β_2 -specific S-ODN's and TGF- β neutralizing antibody on cytotoxicity of PBMC's against autologous cultured glioma cells (target/effector 1 : 10). After 6 days culture of PBMC's with IL-1 α and IL-2 the cells were collected, washed, irradiated (30 Gy) and added in target/effector ratios of 1 : 10, 1 : 5, 1 : 1 to autologous glioma cells. Glioma targets were pretreated with either TGF- β specific S-ODN's or TGF- β antibody. Cytotoxicity was assessed employing a modified microcytotoxicity assay. Data are means of triplicate samples, error bars represents SE. Data points reflect individual controls, where tumor targets were treated with medium alone (control). TGF- β antibody (100 $\mu\text{g}/\text{ml}$), or S-ODN's (1 μM resp. 5 μM) as references for cytotoxicity effects. Thereby, effects upon target cells of antibody or S-ODN's alone could be excluded.

Figure 8: Dose-dependent effects of TGF- β_2 -specific and nonsense S-ODN's on proliferation of lymphocytes, glioma cells and lymphocytes cocultured with autologous glioma cells (MLTC). A: HTZ-153, B: (HTZ-209, C: HTZ-243. PBMC's were preactivated for 6 days with IL-1 α and IL-2 and incubated for additional 6 days with autologous irradiated (60 Gy) and

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TGF- β_2 - (No.6) and nonsense (no. 5) S-ODSN-treated glioma cells (MLTC). Simultaneously, part of preactivated PBMC's (lymphocytes) and glioma cells (tumor) were incubated with TGF- β_2 specific (Ly: No. 2, Tu: No. 4) and nonsense) S-ODN's (Ly: No. 1, Tu: No. 3) for 3 days, to evaluate putative direct effects of S-ODN's upon effector- or target cells alone. Proliferation of lymphocytes and glioma cells was assessed employing a ^3H Tdr incorporation assay. Data are means of triplicate samples, error bars represent SE.

The invention is further explained by the following non-limiting examples.

Example 1

Characterization of tumor cells (autologous target cells)

Tumor cells of 3 patients with high grade malignant gliomas (HTZ-153 and HTZ 209, glioblastomas, HTZ-243, malignant astrocytoma, Gr.III-WHO) and their resp. autologous lymphocytes were studied. Standard tumor cell cultures were established in Dulbecco's Minimal Essential Medium containing 20 % fetal calf serum (FCS, Seromed, Berlin, Germany), 1 μM L-glutamine, MEM vitamin solution and nonessential amino acids (GIBCO, Paisley, Scotland, U. K.) (Bogdahn, U., Fleischer, B., Rupniak, H.T.R., Ali-Osman, F. T-cell mediated cytotoxicity in human glioma Biology of Brain Tumor, Martinus Nijhoff Publishers, Boston, 70: 501 - 507, 1986). Other target cells included K562 (an NK-sensitive erythromyeloid leukemic cell line, American Type Culture Collection, Rockville, MD, USA). Tumor cell cultures were characterized by immunocytochemistry employing the PAP-method (Bourne, J. A., Handbook of immunoperoxidase staining methods, DAKO Corporation, Carpinteria CA, USA, 1983) in Labtek tissue culture slides (Miles Laboratories Inc., Naperville, IL, USA) with the following mono- or polyclonal antibodies to: GFAP, Cytokeratin, Neurofilament, Desmin, Vimentin, NSE, HLA, DrO, W6/32 (Class I Antigen), β_2 -Microglobulin, Fibronectin,

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Laminin, Ki 67 (Dakopatts, Glostrup, Denmark) and anti-TGF- β (R & D Systems, Inc., Minneapolis, MN, USA). TGF- β specific immunocytochemistry was performed after 48 hours incubation of glioma culture slides with 1 μ M final concentration (f.c.) TGF- β_2 -specific S-ODN's and 1 μ M (f.c.) nonsense S-ODN's treated controls.

Example 2

Characterization of lymphocytes (effector cells)

Peripheral blood mononuclear cells from all glioma patients were isolated from heparinized venous blood at the day of surgery, employing Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation and cryopreserved in liquid nitrogen under standard conditions (Bogdahn, U., Fleischer, B., Rupniak, H.T.R., Ali-Osman, F. T-cell mediated cytotoxicity in human glioma Biology of Brain Tumor, Martinus Nijhoff Publishers, Boston, 70: 501 - 507, 1986). Lymphocytes were cultured in RPMI 1640 (Flow Laboratories Inc., Scotland, U.K.) with 10% human pooled AB-serum (Flow Laboratories Inc. McLean, VA, USA) and 2 mM L-glutamine. Native and activated (see below) peripheral blood mononuclear cells were characterized by immunocytochemistry employing alkaline phosphatase and monoclonal anti-alkaline phosphatase complexes (APAAP-method, Dakopatts GmbH, Hamburg, Germany) (Cordell, J. L., Falini, B., Erber, W. N., et al., Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes), J. Histochem. Cytochem., 32: 219 - 229, 1984) with monoclonal antibodies to the following antigens: CD3, CD4, CD8, CD16, CD25, HLA DR (Becton Dickinson, Mountain View, Ca USA).

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Example 3

LAK-cell generation

As the proliferative and cytotoxic response of peripheral blood mononuclear cells from glioma patients is suppressed, cells (2×10^6 cells/ml) were preactivated in vitro for 6 days with interleukin- 1α (10 U/ml). R & D Systems, Inc., Minneapolis, MN, USA) and interleukin-2 (100 U/ml), BIOTEST AG Frankfurt/M. Germany) in 48 flat bottom tissue culture plates (2×10^6 cells/ml) (Costar, Cambridge, MA, USA).

Example 4

Proliferation assay

In mixed lymphocyte-tumor cell cultures (MLTC) 15×10^3 lethally irradiated (60 Gy, ^{64}Co -source) tumor cells served as stimulators, and were cocultivated with 25×10^3 pre-activated mononuclear cells (LAK-cells, see above) for 6 days in 96-well-flat bottom tissue culture plates (NUNC, Copenhagen, Denmark). In MLTC-experiments, the same culture medium conditions were employed as during preactivation. In antisense experiments, TGF- β_2 -specific phosphorothioate oligodeoxynucleotides (S-ODN's) and nonsense oligodeoxynucleotides (see below) were added to the cultures 12 hours before MLTC assay. Anti-TGF- β neutralizing antibodies (R & D Systems, Inc. Minneapolis, MN, USA) were added to the culture 2 hours before MLTC.

Example 5

Cytotoxicity assay

Cytotoxicity experiments were performed with a modified microcytotoxicity assay (Bogdahn, U., Fleischer, B., Rupniak, H.T.R., Ali-Osman, F. T-cell mediated cytotoxicity in human

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glioma Biology of Brain Tumor, Martinus Nijhoff Publishers, Boston, 70: 501 - 507, 1986). Briefly, 1.5×10^3 target cells were seeded into 96-well flat bottom tissue culture plates. Twelve hours after plating, TGF- β_2 -specific S-ODN's and nonsense oligodeoxynucleotides (anti-sense-controls) were added to the culture. Anti-TGF- β neutralizing antibodies and normal rabbit serum (antibody-controls, R & D Systems, Inc. Minneapolis, MN, USA) were added to the culture 22 hours after plating. Various ratios (target/effector ratio of 1 : 1, 1 : 5, 1 : 10 of preactivated effector cells (LAK-cells) were irradiated (30 Gy), and added to respective targets 24 hours after plating for 3 days under standard culture conditions (RPMI 1640 culture medium containing 10 % pooled AB-serum and 2 μ M L-Glutamine). No cytokines were added to the culture during cytotoxicity experiments. An incubation period of 3 days was selected, as statistical evaluation of data turned out to be optimal at this time point. Killing of target cells was demonstrated by incorporation of Trypan blue dye (data not presented). Target cell proliferation in LAK-cell treated targets) was assessed with a standard ^3H -Thymidine incorporation assay (6- ^3H -Thymidine, 1 $\mu\text{Ci}/\text{well}$, spec. Activity 27 Ci/mmol). Liquid scintillation counting of ^3H -thymidine incorporation was performed after 18 hours of incubation of cells. The specific cytotoxicity was calculated as:

$$(\text{cpm}_{(\text{control})} - \text{cpm}_{(\text{probe})} / \text{cpm}_{(\text{control})}) \times 100\%.$$

Example 6

Northern and Western blot analysis

Cytoplasmatic RNA was prepared by lysing glioma cells treated with 1 μM (f.c.) TGF- β_2 -specific S-ODN's for 48 hours and untreated controls in buffer containing 0.5 % NP-40 (Sambrook, J., Fritsch, E. F., Maniatis, T. Molecular cloning. A laboratory manual, 2nd Edition, Cold Spring Harbor

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Laboratory Press, 1989). For Northern hybridization aliquots of 20 μ g denaturated RNA were separated by electrophoresis on 1% agarose-formaldehyd gel. The quality and quantity of immobilized RNA was verified by methylene-blue staining of the Hybond-N membranes (Amersham/Buchler, Braunschweig, Germany) after transfer. Blots were hybridized overnight with specific TGF- β_1 - or TGF- β_2 -synthetic oligonucleotide probes (40-mer, Oncogen Science, Seattle, USA), 5' labeled with (gamma- 32 P)-ATP employing T4 polynucleotide kinase (Pharmacia, Freiburg, Germany) and exposed to X-ray film.

For Western blotting, TGF- β -S-ODN treated (48 hours, 1 μ M f. c.) resp. untreated glioma cells were grown in medium containing 10 % FCS washed and further cultured in defined serum free medium for 24 hours. The cells were lysed employing a lysis buffer containing NP-40. 30 μ g of total cellular protein were loaded onto each lane of a 12 % polyacrylamide-SDS gel. Fractionated proteins were then electroblotted to a nitrocellulose membrane for 20 minutes at 0.8 mA/cm² as described (Towbin, H., Staehelin, T., Gordon, J. Electrophoretic transfer of proteins from PAGE to nitrocellulose sheets: procedure and some applications, Proc. Natl. Acad. Sci., USA, 76: 4350 - 4354, 1979). Filters were probed with a polyclonal antibody of TGF- β_2 (R & D Systems Inc. Minneapolis, USA) 50 μ g of TGF- β served as control.

Example 7

Phosphorothioate modified antisense oligodeoxynucleotides (S-ODN's)

TGF- β_2 -specific antisense oligodeoxynucleotides (antisense direction of TGF- β_2 mRNA primer sequene oligonucleotide seequence: CAGCACACAGTACT) and randomized nonsense sequence with the same GC-content as the specific S-ODN's (nonsense oligonucleotide sequence: GTCCCTATACGAAC) were synthesized

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on an Applied Biosystems model 380 B DNA Synthesizer (Schlingensiepen, K.-H., Brysch, W. Phosphorothioate oligomers. Inhibitors of oncogene expression in tumor cells and tools for gene function analysis in : Erikson, R., Izant., J. (Eds.) Gene regulation by antisense nucleic acids. Raven Press New York 1992). S-ODN's were removed from the solid support with 33 % ammonnia. Oligonucleotides still bearing the 5' trityl protecting group were purified by reverse phase HPLC, with an Aquapore RP-300, C8-column(Brownlee). Solvents: A - 0.1 M TEAA pH 7, B-Acetonitrile. Gradient 3 - 35 % B over 30 Min. linear. Trityl bearing fraction of oligonucleotides, corresponding to the full-length product were detritylated in 80% acetic acid/ETOH for 20 Min. extracted twice with diethyl-ether, desalted on a Sephadex G 25 (Pharmacia) column, ethanol precipitated (2x) and finally diluted in 0.1 M Tris/HCL pH 7.6. S-ODN's were judged from polyacrylamid-gel-electrophoresis to be more than 85 % full-length material.

Example 8

Characterization of tumor cells

All glioma cell cultures expressed GFAP, TGF- β , vimentin, and HLA-Class I antigens, as well as β -microglobulin, fibronectin, and KI 67, inconsistent expression was found with desmin, HLA-Class II antigen (positive: HTZ-209) and NSE (positive: HTZ-209, HTZ-243). No expression was found for cytokeratin, laminin and neurofilaments, indicating the glial origin of these tumor cells.

Western blot analysis of tumor cell lysates revealed that HTZ-153, HTZ-209 and HTZ-243 cells produced TGF- β_2 protein (Fig. 3).

Northern blot analysis of cytoplasmatic RNA's from all 3 tumors revealed message for TGF- β_1 (2.3 kB) and TGF- β_2 (4.1 kB) (Fig. 5 and 5): message for TGF- β_1 was fairly well

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represented in all three tumors (Fig. 4), however, tumor HTZ- 209 displayed a faint TGF- β_2 signal compared to the remaining tumors (Fig. 5).

Example 9

Modulation of TGF- β expression by treatment of glioma cells with TGF- β_2 specific S-ODN's

The effects of TGF- β_2 -specific S-ODN-treatment upon TGF- β_2 mRNA- and -protein expression in glioma cells were analysed by Northern blotting. Western Blotting and immunocytochemistry. Northern blot analysis of glioma cells treated with TGF- β_2 -specific S-ODN's (f.c. 1 μ M for 48 hours) yielded inconsistent results: HTZ-153 displayed an increase in TGF- β_2 -message, whereas tumors HTZ-209 and HTZ-243 showed no detectable message following antisense oligodeoxynucleotides treatment (Fig. 6). Western blot analysis revealed a decreased TGF- β_2 -specific signal for all 3 tumors after S-ODN treatment (Fig. 3).

Immunostaining of glioma cultures treated with TGF- β_2 -specific S-ODN's (f.c. 1 μ M for 48 hours) revealed a decrease of TGF- β -dependant immunoreactivity compared to nonsense S-ODN-treated and untreated controls for all 3 tumors. Controls with normal mouse serum and human AB-serum were negative (slides not presented).

Example 10

Characterization of lymphocytes

Autologous effector lymphocytes employed in the following experiments on tumor dependant lymphocyte proliferation and glioma cytotoxicity were characterized by conventional lymphocyte differentiation antigens. Data of characterization experiments are displayed in table 1, cell populations

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reflect the phenotype of lymphocyte subsets of native (Day 0) and activated (Day 6) effector cells, employed in proliferation and cytotoxicity experiments. The percentage of CD3⁺ cells increased during culture time, up to 85 %. The same was true for CD4⁺ (up to 80 %). CD8⁺ (up to 18), CD25⁺ (up to 60%)-cells, the fraction of CD16⁺ cells increased to a maximum of 50% (HTZ-243) during the first 6 days of culture.

Example 11

Cytotoxicity experiments

Native PBMC's of tumor-patients investigated in our study expressed low cytotoxic activity to autologous targets, (below 20% at target/effector ration 1 : 10. Preliminary experiments disclosed that preactivation of autologous effector PBMC's was most effective, when cells were incubated with 10 U/ML IL-1 α adn 100 U/ml IL-2 for 6 days. These LAK-cells were employed in all further cytotoxicity/proliferation experiments.

At a target/effector ration of 1/10, LAK cells achieved a cytotoxic activity of up to 25% in the autologous target systems (Fig. 7). Preincubation of tumor cells with neutralizing TGF- β antibodies (f.c. 100 μ g/ml) resulted in a cytotoxicity of 30% - 50% (5 - 30% increase above the untreated controls) (Fig. 7). When tumor cells were pre-incubated with TGF- β_2 -specific antisense S-ODN's cytotoxicity increase in a dose dependent fashion to a maximum of 79% (5 μ M S-ODN's, 25 - 60% increase above untreated controls) and 67% (1 μ M S-ODNs, 15 - 45% increase above untreated autologous lymphocytes. All three effector cell populations expressed high NK-activity as detected by cytotoxicity assay against K 562 cell line, ranging from 60% to 75%.

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Example 12

Proliferation experiments

Lymphocyte proliferation upon stimulation with autologous tumor cells (MLTC) treated with TGF- β_2 -specific S-ODNs was increased in tumors HTZ-153 (Fig. 8a) and HTZ-209 (Fig. 8b), however, no effect was observed in HTZ-243 cells (Fig. 8c). Nonsense S-ODN's at a final concentration (f.c.) of 1 μ M did not alter lymphocyte proliferation (Fig. 8). Effects of TGF- β_2 -specific S-ODN's were observed in a dose dependant fashion from 0.1 μ M up to 1 μ M, higher concentrations (5 μ M) displayed non-specific toxicity towards PBMC's and tumor cells (Fig. 8): the proliferation of PBMC's in S-ODN treated MLTC's and tumor cells (Fig. 8): the proliferation of PBMC's in S-ODN treated MLTC's was persistently lower for oligonucleotide concentrations above 1 μ M. High concentrations of neutralizing TGF- β antibody (100 μ g/ml) did not enhance lymphocyte proliferation. TGF- β_2 -specific antisense S-ODN's had an inhibitory effect upon proliferation of either cultured lymphocyte populations (marginal effect) or autologous target cells (Fig. 8) achieving a maximum of 75 % at a S-ODN's concentration of 5 μ M (f.c.). Less profound inhibitory effects were observed with randomized control nonsense S-ODN's (average 20%, up to 40% at 5 μ M f.c.).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT

- (A) NAME: Biognostik Gesellschaft fuer biomolekulare Diagnostik mbH
- (B) STREET: Carl-Giesecke-Str. 3
- (C) CITY: Goettingen
- (E) COUNTRY: Germany
- (F) POSTAL CODE (ZIP): 37079

(ii) TITLE OF INVENTION: Antisense-oligonucleotides for the treatment of immuno-suppressive effect of transforming-growth-factor beta (TGF-beta)

(iii) NUMBER OF SEQUENCES: 137

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0 Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCAGGTGGA TAGT

14

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATGTCGATA GTCTTGCA

18

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCGATAGTC TTGC

14

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCATGTCGAT AGTC

14

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCCATGTCG ATAG

14

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTTGGACAGG ATCT

14

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCTGTTGTA CAGG

14

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTGCTGTTGT ACAG

14

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTGCGTAGT AGTC

14

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(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCCACCATTA GCAC

14

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GATTTCGTTG TGGG

14

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTCATAGATT TC GTTGTG

18

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TGTACTCTGC TTGAAC

16

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGTACTCTG CTTG

14

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGCTGTGTGT ACTC

14

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGATGTGTT GAAGAACA

18

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(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTCTGATGTG TTGAAG

16

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCTCTGATGT GTTG

14

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAGCTCTGAT GTGT

14

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CACTTTTAAC TTGAGCCT

18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTCCACTTTT AACTTGAG

18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGCTGTATTT CTGGTACA

18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCAGGAATTG TTGC

14

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(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TTGCTGAGGT ATCG

14

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GATAACCACT CTGG

14

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CAAAAGATAA CCACTCTG

18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
CGGTGACATC AAAAG 15

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
CCTCAATTTC CCCT 14

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GTTATCCCTG CTGT 14

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GCAGTGTGTT ATCC 14

- 33 -

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GATGTCCACT TGCA

14

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TAGTGAACCC GTTG

14

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TGCCATGAAT GGTG

14

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTTTCATGCCA TGAATG

16

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CATGAGAAGC AGGA

14

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCTTTGCAGA TGCT

14

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GAGCTTTGCA GATG

14

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(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TAGTTGGTGT CCAG

14

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTGAAGCAAT AGTTGG

16

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGCTGAAGCA ATAGTTGG

18

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGAGCTGAAG CAAT

14

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CAATGTACAG CTGC

14

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GGAAGTCAAT GTACAG

16

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CGGAAGTCAA TGTAC

15

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(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCGGAAGTCA ATGT

14

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

AGTTGGCATG GTAG

14

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCAGAAGTTG GCAT

14

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

CTCCAAATGT AGGG

14

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

ACCTTGCTGT ACTG

14

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

TGCTGGTTGT ACAG

14

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GGTTATGCTG GTTG

14

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(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GTAGTACACG ATGG

14

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CGTAGTACAC GATG

14

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

CACGTAGTAC ACGA

14

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CATGTTGGAC AGCT

14

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GCACGATCAT GTTG

14

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CACACAGTAG TGCA

14

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GATCAGAAAA GCGC

14

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(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

ACCGTGACCA GATG

14

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GTAGACAGGC TGAG

14

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TATCGAGTGT GCTG

14

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TTGCGCATGA ACTG

14

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TTGCTCAGGA TCTG

14

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

ACTGGTGAGC TTCA

14

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

ATAGTCTTCT GGGG

14

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(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GCTCAGGATA GTCT

14

(2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

TG TAGATGGA AATCACCT

18

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TGGTGCTGTT GTAG

14

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

TTCTCCTGGA GCAA

14

(2) INFORMATION FOR SEQ ID NO:70:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

TACTCTTCGT CGCT

14

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

CTTGCGGTAG TACT

14

(2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

CGGCATGTCT ATTTTGTA

18

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(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

TTTTCGGAGG GGAA

14

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CGGGATGGCA TTTT

14

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

CTGTAGAAAG TGGG

14

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:
ACAATTCTGA AGTAGGGT 18
- (2) INFORMATION FOR SEQ ID NO:77:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:
ATTGCTGAGA CGTCAAAT 18
- (2) INFORMATION FOR SEQ ID NO:78:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:
TCTCCATTGC TGAG 14
- (2) INFORMATION FOR SEQ ID NO:79:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:
TCACCAAATT GGAAGCAT 18

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(2) INFORMATION FOR SEQ ID NO:80:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

CTCTGAACTC TGCT

14

(2) INFORMATION FOR SEQ ID NO:81:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

AACGAAAGAC TCTGAACT

18

(2) INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

TGGGTTCTGC AAAC

14

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CTGGCTTTTG GGTT

14

(2) INFORMATION FOR SEQ ID NO:84:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GTTGTTTCAGG CACT

14

(2) INFORMATION FOR SEQ ID NO:85:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TCTGATATAG CTCAATCC

18

(2) INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

TCTTTGGACT TGAGAATC

18

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(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TGGGTTGGAG ATGT

14

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

TGCTGTTCGAT GTAG

14

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

ACAACTTTGC TGTCGA

16

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:
ATTCGCCTTC TGCT 14

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:
GAAGGAGAGC CATT 14

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:
TCAGTTACAT CGAAGG 16

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:
TGAAGCCATT CATGAACA 18

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(2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TCCTGTCTTT ATGGTG

16

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

AAATCCCAGG TTCC

14

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GGACAGTGTA AGCTTATT

18

(2) INFORMATION FOR SEQ ID NO:97:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GTACAAAAGT GCAGCA

16

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

TAGATGGTAC AAAAGTGC

18

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

CACTTTTATT TGGGATGATG

20

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GCAAATCTTG CTTCTAGT

18

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(2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GTGCCATCAA TACC

14

(2) INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GGTATATGTG GAGG

14

(2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TCTGATCACC ACTG

014

(2) INFORMATION FOR SEQ ID NO:104:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:
TCCTAGTGGA CTTTATAG 18

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:
TTTTTCCTAG TGGACT 16

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:
AGATGTGGGG TCTT 14

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:
CAATAACATT AGCAGG 16

- 55 -

(2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

AAGTCTGTAG GAGG

14

(2) INFORMATION FOR SEQ ID NO:109:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

TCTGTTGTGA CTCAAG

16

(2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

GTTGGTCTGT TGTG

14

(2) INFORMATION FOR SEQ ID NO:111:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

CAAAGCACGC TTCT

14

(2) INFORMATION FOR SEQ ID NO:112:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

TTTCTAAAGC AATAGGCC

18

(2) INFORMATION FOR SEQ ID NO:113:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

GCAATTATCC TGCACA

16

(2) INFORMATION FOR SEQ ID NO:114:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

ACGTAGGCAG CAAT

14

- 57 -

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

ATCAATGTAA AGTGGACG

18

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

CTAGATCCCT CTTG

14

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

CCATTTCAC CTA

14

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

TGGGTTTCGTG TATC

14

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TGGCATTGTA CCCT

14

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

TCCAGCACAG AAGT

14

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

ATAAATACGG GCATGC

16

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(2) INFORMATION FOR SEQ ID NO:122:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

AGTGTCTGAA CTCC

14

(2) INFORMATION FOR SEQ ID NO:123:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

TGTGCTGAGT GTCT

14

(2) INFORMATION FOR SEQ ID NO:124:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

ATAAGCTCAG GACC

14

(2) INFORMATION FOR SEQ ID NO:125:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:
AGGAGAAGCA GATG 14

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:
AGCAAGGAGA AGCA 14

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:
AATCTTGGA CACG 14

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:
TAGAGAATGG TTAGAGGT 18

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(2) INFORMATION FOR SEQ ID NO:129:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

GTTTTGCCAA TG TAGTAG

18

(2) INFORMATION FOR SEQ ID NO:130:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

CTTGGGTGTT TTGC

14

(2) INFORMATION FOR SEQ ID NO:131:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

GCAAGACTTT ACAATC

16

(2) INFORMATION FOR SEQ ID NO:132:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

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(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

GCATTTGCAA GACTTTAC

18

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

TTTAGCTGCA TTTGCAAG

18

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

GCCACTTTTC CAAG

14

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: unknown
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

TTGGTCTTGC CACT

14

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(2) INFORMATION FOR SEQ ID NO:136:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

CAGCACACAG TAGT

14

(2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 14 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

CGATAGTCTT GCAG

14

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C l a i m s

1. Antisense-oligonucleotides or effective derivatives thereof hybridizing with an area of a gene coding for transforming growth factor- β (TGF- β) comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID NO. 1 - 56 and 137 or comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID NO. 57 to 136 each of the nucleic acids having a DNA- or RNA-type structure.
2. Antisense-oligonucleotides according to claim 1 wherein said nucleic acid hybridizes with an area of a gene coding for transforming growth factor- β_1 , - β_2 and/or - β_3 .
3. Antisense-oligonucleotides according to claim 1 and/or 2 wherein said nucleic acid is hybridizing with areas of a gene region coding for TGF- β and/or areas of a gene region coding and non-coding for TGF- β .
4. Antisense-oligonucleotide according to any one of the claims 1 - 3 wherein the antisense-oligonucleotide is a phosphorothioate oligodeoxynucleotide.
5. Antisense-oligonucleotide according to any one of the claims 1 to 4 obtainable by solid phase synthesis using phosphite triester chemistry by growing the nucleotide chain in 3'-5' direction in that the respective nucleotide is coupled to the first nucleotide which is covalently attached to the solid phase comprising the steps of

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- cleaving 5' DMT protecting group of the previous nucleotide,
 - adding the respective nucleotide for chain propagation,
 - modifying phosphite groups subsequently cap unreacted 5'-hydroxyl groups and
 - cleaving the oligonucleotide from the solid support,
 - followed by working up the synthesis product.
6. Antisense-oligonucleotide according to any one of the claims 1 to 5 wherein the oligonucleotides of the SEQ ID NO. 1 to 56 and 137 are antisense-oligodeoxynucleotides of TGF- β_1 and oligonucleotides of the SEQ ID NO. 57 to 136 are phosphorothioate-antisense-oligodeoxynucleotide of TGF- β_2 .
7. Oligonucleotide as nonsense-control-nucleotide with an identical amount of GC as the antisense-oligonucleotide according to any one of the claims 1 to 6.
8. Pharmaceutical composition comprising an antisense-nucleic acid according to any one of the claims 1 to 6.
9. Use of antisense-oligonucleotides according to any one of the claims 1 to 6 for the manufacturing of a pharmaceutical composition of claim 8 for the treatment of tumors in which expression of TGF- β is of relevance for pathogenicity and/or inhibition of pathological angiogenesis.
10. Use according to claim 9 for manufacturing pharmaceutical composition for the treatment of the immunosuppressive effect of TGF- β , augmentation of the proliferation of cytotoxic lymphocytes, for the treat-

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ment of endogenous hyper expression of TGF- β , for treatment of breast tumors, for treatment of neurofibroma, malignant glioma including glioblastoma for the treatment and prophylaxis of skin carcinogenesis as well as treatment of esophageal and gastric carcinomas.

11. Method of treating tumors in which the expression of TGF- β is of relevance for pathogenicity by inhibiting TGF- β and thereby reducing an immuno suppression and/or inhibition of pathological angiogenesis by administration of an effective dose of an antisense-TGF- β -oligonucleotides according to any one of the claims 1 to 6.
12. Method according to claim 11 wherein the reduction of immuno suppression is accompanied by an augmented proliferation of cytotoxic lymphocytes in comparison with the status before administration of the antisense-TGF- β -oligonucleotides and thereupon starting cytotoxic activity decreases the numbers of tumor cells.

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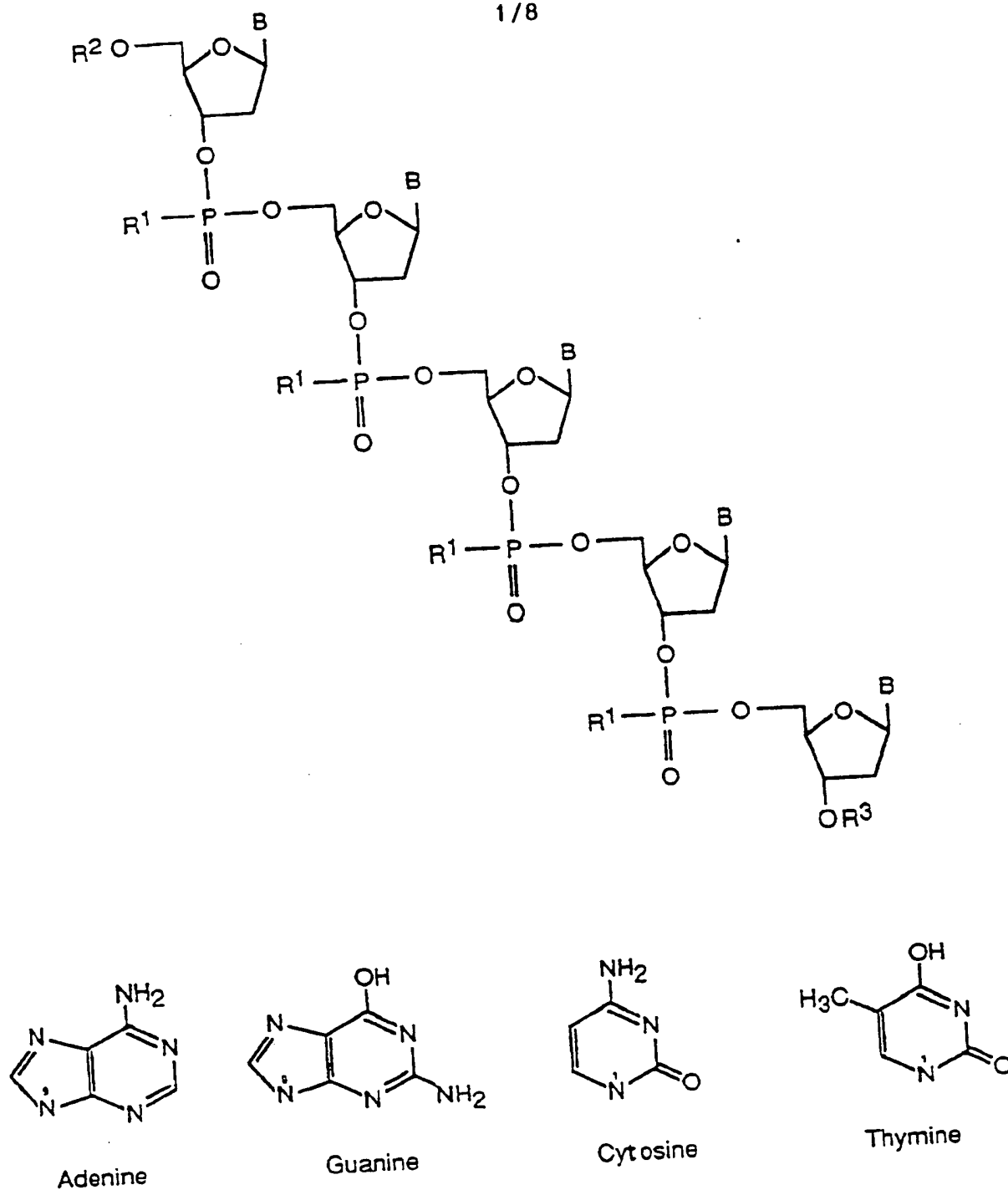
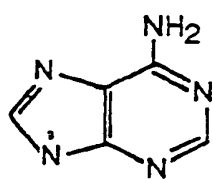
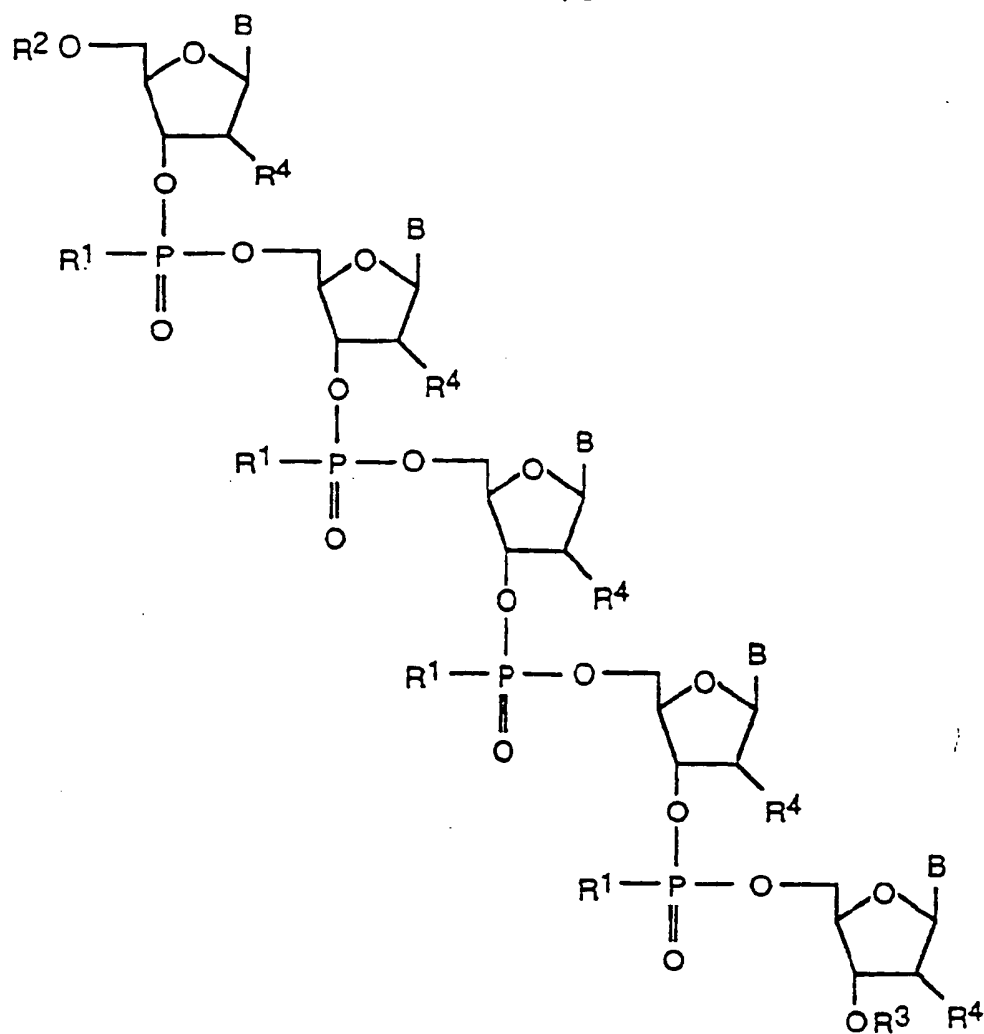
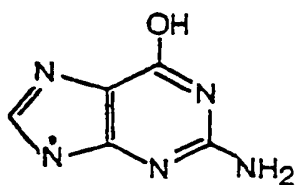


FIG 1

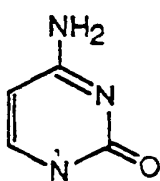
2/8



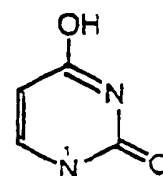
Adenine



Guanine



Cytosine



Uracil

FIG. 2

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Fig. 3

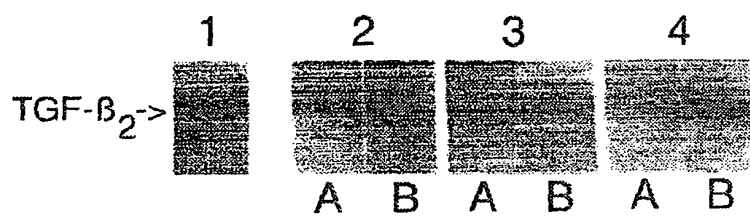
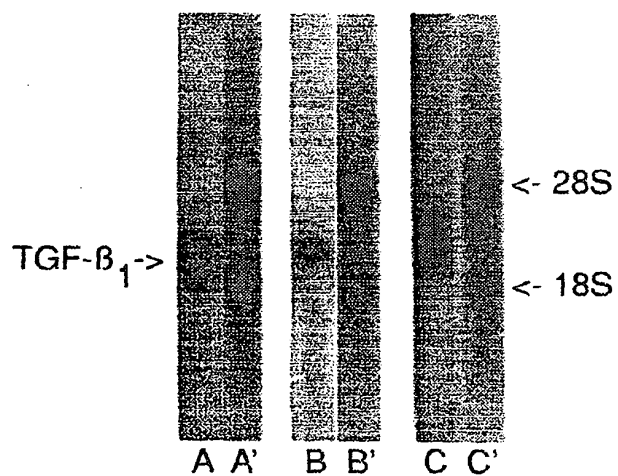


Fig. 4



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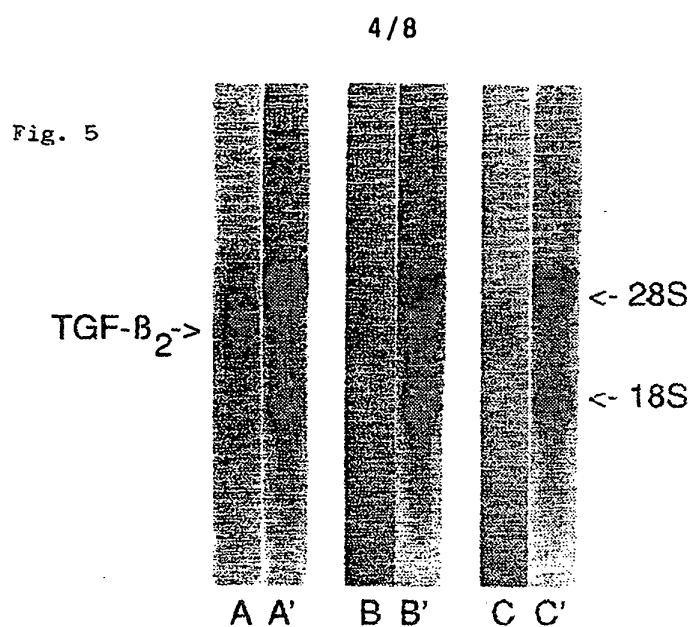
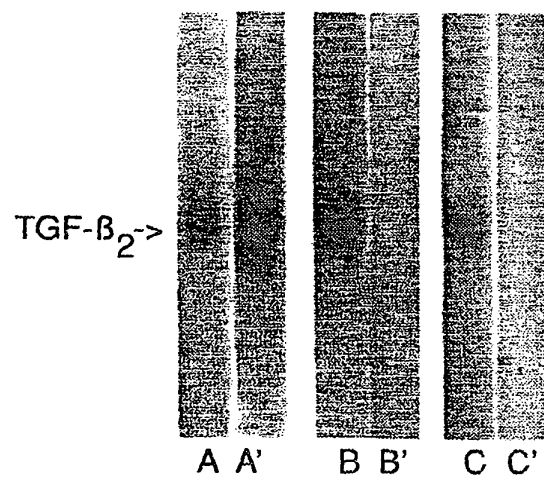
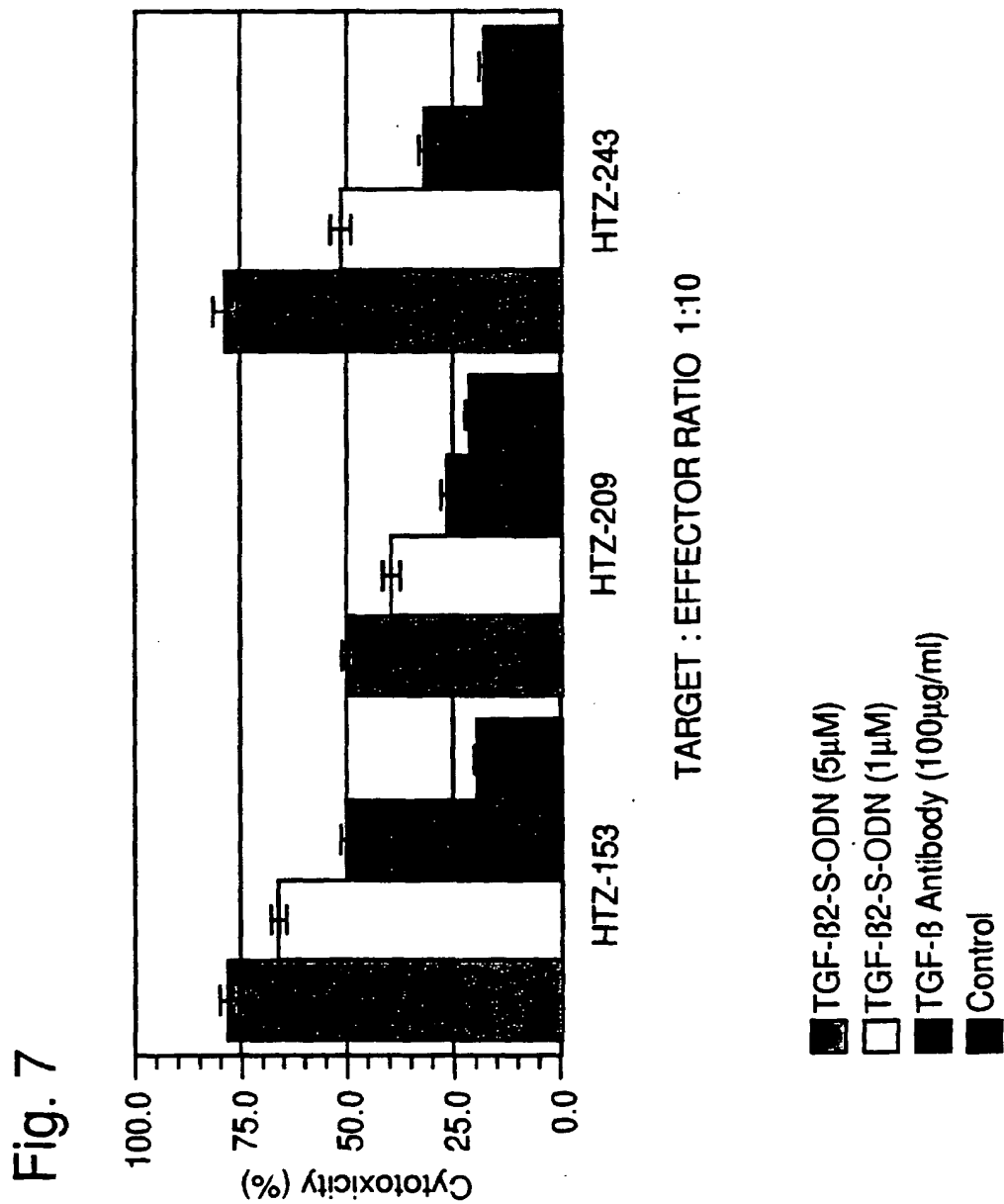


Fig. 6

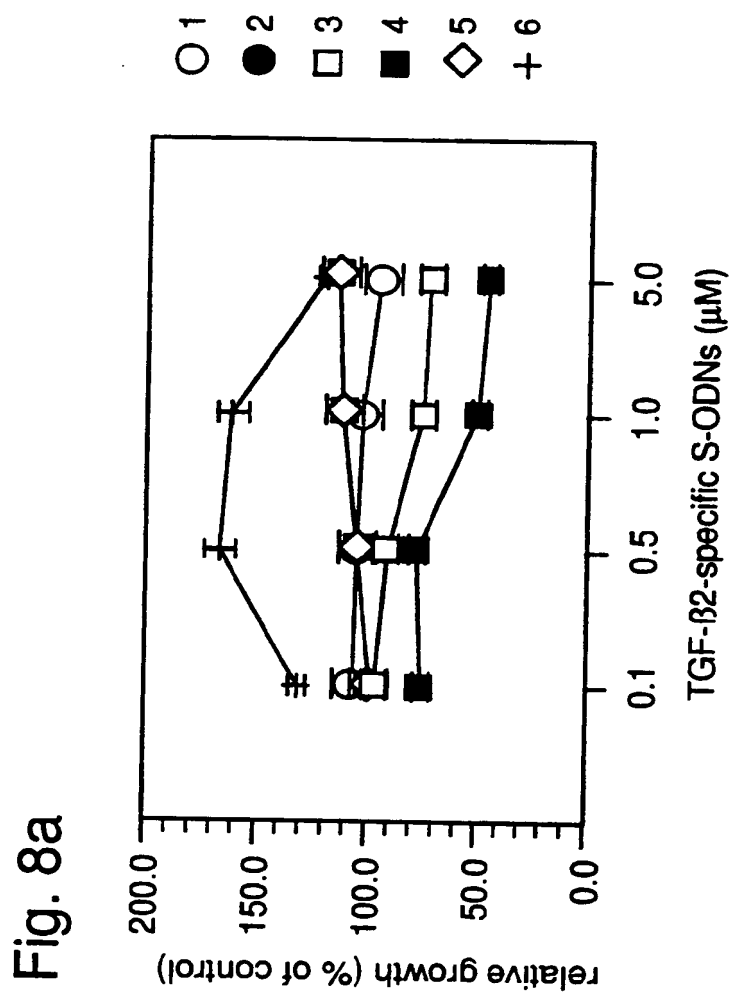


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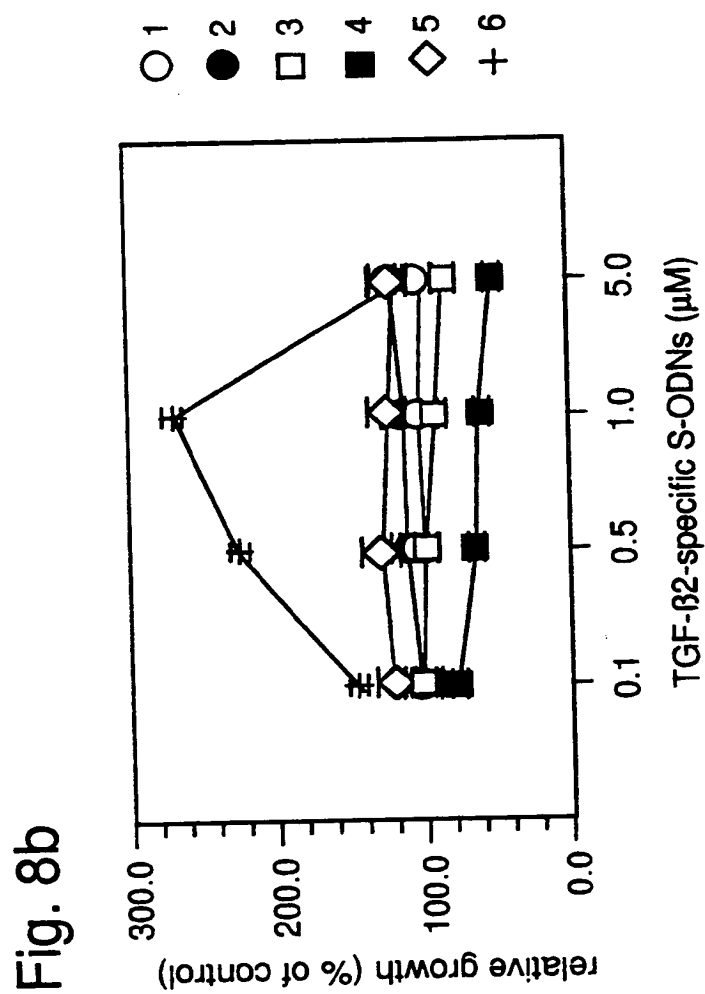


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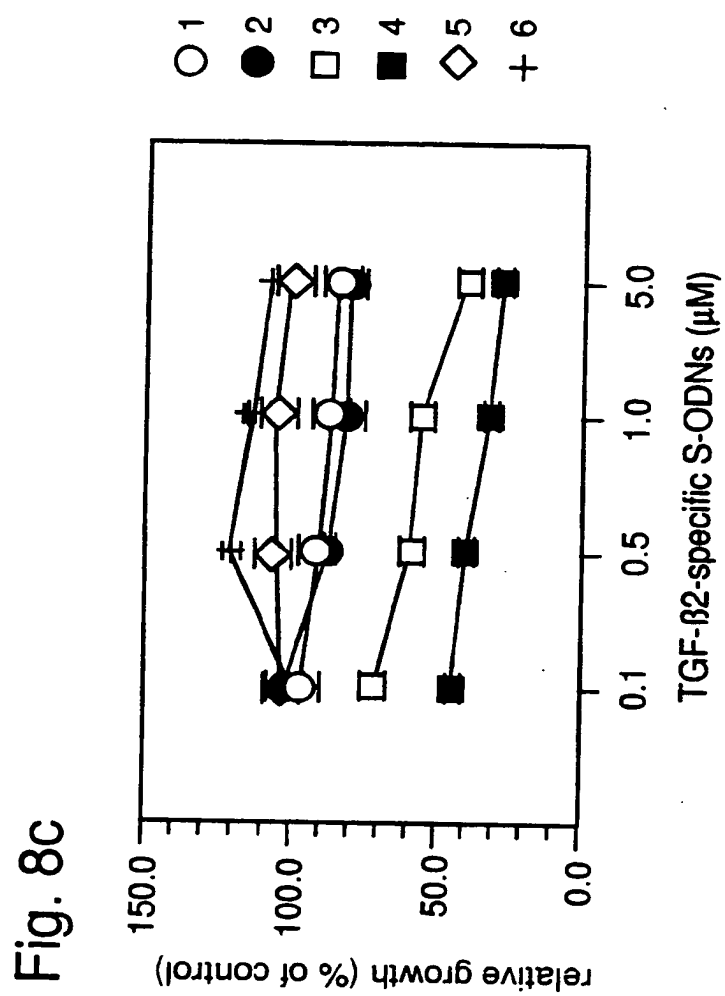
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(21) International Application Number: PCT/EP94/01362 (22) International Filing Date: 29 April 1994 (29.04.94) (30) Priority Data: 93 107 089.0 30 April 1993 (30.04.93) EP (34) Countries for which the regional or international application was filed: DE et al. 93 107 849.7 13 May 1993 (13.05.93) EP (34) Countries for which the regional or international application was filed: DE et al. (71) Applicant (for all designated States except US): BIOGNOSTIK GESELLSCHAFT FÜR BIOMOLEKULARE DIAGNOSTIK MBH [DE/DE]; Carl-Giesecke-Strasse 3, D-37079 Göttingen (DE). (72) Inventors; and (75) Inventors/Applicants (for US only): SCHLINGENSIEPEN, Georg-Ferdinand [DE/DE]; Am Goldgraben 20, D-37073 Göttingen (DE). BRYSCH, Wolfgang [DE/DE]; Am Goldgraben 2, D-37073 Göttingen (DE). SCHLINGENSIEPEN, Karl-Hermann [DE/DE]; Bovender Strasse 5, D-37120 Bovenden (DE). SCHLINGENSIEPEN, Reimar [DE/DE]; Am Goldgraben 13, D-37073 Göttingen (DE). BOGDAHN,		Ulrich [DE/DE]; Mittlere Heerbergstrasse 25a, D-97078 Würzburg (DE). (74) Agents: MEYERS, Hans-Wilhelm et al.; Deichmannhaus am Hauptbahnhof, D-50667 Köln (DE). (81) Designated States: AU, CA, CN, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 27 October 1994 (27.10.94)
(54) Title: ANTISENSE-OLIGONUCLEOTIDES FOR THE TREATMENT OF IMMUNOSUPPRESSIVE EFFECTS OF TRANSFORMING GROWTH FACTOR- β (TGF- β) (57) Abstract Antisense-oligonucleotides or effective derivatives thereof hybridizing with an area of a gene coding for transforming growth factor- β (TGF- β) comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID No. 1 - 56 and 137 or comprising the following nucleic acid sequences identified in the sequence listing under SEQ ID No. 57 to 136 each of the nucleic acids having a DNA- or RNA-type structure.		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/94/01362

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 C12N15/11 A61K31/70 C07H21/00 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N A61K C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,0	PROCEEDINGS OF THE 82ND ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, HOUSTON, TEXAS, USA, MAY 15-18, 1991. PROC AM ASSOC CANCER RES ANNU MEET 32 (0). 1991. 248 JACHIMCZAK, P. ET AL 'TGF-BETA-PHOSPHOROTHIOATE-ANTISENSE OLIGONUCLEOTIDES MAY REVERSE IMMUNOSUPPRESSIVE EFFECTS OF TGF-BETA IN MALIGNANT GLIOMAS IN VITRO' * abstract 1474 * --- -/--	1-5,9-12



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

17 August 1994

Date of mailing of the international search report

08 -11- 1994

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 94/01362

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, O	<p>PROCEEDINGS OF THE 82ND ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, HOUSTON, TEXAS, USA, MAY 15-18, 1991. PROC AM ASSOC CANCER RES ANNU MEET 32 (0). 1991. 427.</p> <p>BEHL C et al 'TRANSFORMING GROWTH FACTOR - BETA - ANTISENSE -OLIGONUCLEOTIDES INHIBIT A HUMAN MELANOMA CELL LINE UNDER SERUM-ENRICHED AND STIMULATE UNDER SERUM-FREE CULTURE CONDITIONS.'</p> <p>cited in the application</p> <p>* abstract 2541 *</p> <p>---</p>	1-5, 9-12
A	<p>J EXP MED 174 (4). 1991. 925-930.</p> <p>HATZFIELD J et al 'RELEASE OF EARLY HUMAN HEMATOPOIETIC PROGENITORS FROM QUIESCENCE BY ANTISENSE TRANSFORMING GROWTH FACTOR BETA -1 OR RB OLIGONUCLEOTIDES.'</p> <p>cited in the application</p> <p>see the whole document</p> <p>---</p>	1-5, 7, 11, 12
A	<p>JOURNAL OF IMMUNOLOGY,</p> <p>vol.143, no.10, 15 November 1989,</p> <p>BALTIMORE US</p> <p>pages 3222 - 3229</p> <p>BODMER, S. ET AL. 'Immunosuppression and transforming growth factor-beta in glioblastoma'</p> <p>cited in the application</p> <p>see the whole document</p> <p>---</p>	9-12
A	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,</p> <p>vol.88, February 1991, WASHINGTON US</p> <p>pages 1516 - 1520</p> <p>POTTS, J ET AL. 'Epithelial-mesenchymal transformation of embryonic cardiac endothelial cells is inhibited by modified antisense oligodeoxynucleotide to transforming growth factor beta3'</p> <p>cited in the application</p> <p>see table 1</p> <p>-----</p>	1-3, 5

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 94/01362

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

See annex
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See annex

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1 - 12 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

THE APPLICATION CONTAINS FOLLOWING INVENTIONS:

1) Claims: 1-12 (all partially)

Antisense oligonucleotides hybridizing to TGF-beta 1 mRNA or DNA, their use in the preparation of pharmaceutical compositions for the treatment of tumors where the expression of TGF-beta 1 is of relevance.

2) Claims: 1-12 (all partially)

Antisense oligonucleotides hybridizing to TGF-beta 2 mRNA or DNA, their use in the preparation of pharmaceutical compositions for the treatment of tumors where the expression of TGF-beta 2 is of relevance.

3) Claims: 1-12 (all partially)

Antisense oligonucleotides hybridizing to TGF-beta 3 mRNA or DNA, their use in the preparation of pharmaceutical compositions for the treatment of tumors where the expression of TGF-beta 3 is of relevance.

"Remark: Although claims 11-12 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition."